# Pancytopenia related to azathioprine – an enzyme deficiency caused by a common genetic polymorphism: a review

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## Introduction

Azathioprine has been used extensively for immunosuppression in autoimmune disease and organ transplantation for over 25 years. Despite extensive clinical experience much of the drug's pharmacodynamic and pharmacokinetic characteristics are unknown; its mode of action at the immune cell level remains unclear.

The azathioprine structure was synthesized to protect the sulphydryl group of 6-mercaptopurine (6-MP) from in vivo methylation but, subsequent studies have shown that after an oral dose azathioprine is rapidly converted to 6-MP[1,2] which in turn undergoes extensive metabolism along three competing routes (Figure 1). In vivo methylation is a major catabolic pathway catalysed by the enzyme thiopurine methyltransferase (TPMT). Wide interindividual differences in TPMT activity exist which are controlled by a common genetic polymorphism[3]. Eighty nine per cent of the population have high and 11% intermediate enzyme activities whilst one in 300 subjects has very low or absent TPMT activity. A second catabolic route is oxidation catalysed by xanthine oxidase. Measurements of functional xanthine oxidase activity have shown that there is little interindividual or interethnic variation of enzyme activity in the normal population[4], whilst direct measurements of hepatic xanthine oxidase activities in biopsy tissue report a 3.9-fold variation[5].

Active nucleotide metabolites are produced by the third route which is catalysed by the enzyme hypoxanthine phosphoribosyl transferase. This pathway is responsible for the formation of several intracellular active metabolites. The major active metabolites in the human red blood cell (RBC) are the 6-thioguanine nucleotides (6-TGN). Active nucleotide metabolites of azathioprine, which include 6-TGN, are responsible for both cytotoxicity and the suppression of de novo purine synthesis. Both mechanisms contribute to the immunosuppressive properties of azathioprine but, in vitro evidence suggests that this may not be the sole mechanism of action[6].

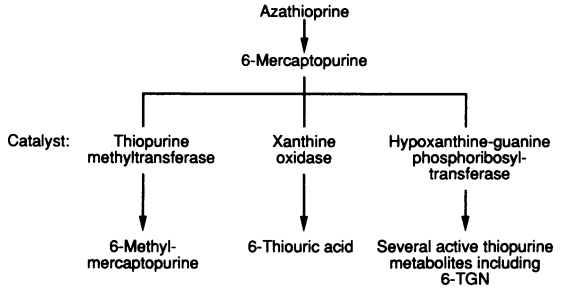


*Figure 1. Metabolism of azathioprine; main metabolic routes*

We have reviewed the literature and identified cases of severe myelosuppression associated with azathioprine. In addition we report a further case of acute and profound bone marrow suppression which developed shortly after onset of treatment with azathioprine. This was associated with abnormally high concentrations of the azathioprine cytotoxic metabolites 6-TGNs and a very low inherited level of the enzyme TPMT. This is the second case we have seen of early and profound myelosuppression associated with azathioprine used in conventional doses for the treatment of oral lichen planus. The first case has previously been reported[7,8].

## Correlation of acute bone marrow failure following azathioprine with deficiency in thiopurine methyl transferase

The clinical relevance of TPMT deficiency to patients treated with azathioprine was reported by Lennard *et al.*[8] who described five patients with acute bone marrow failure after short courses of azathioprine, all with extremely low TPMT activity. Red blood cell 6-TGN levels were very high, confirming the inverse relationship between RBC 6-TGN concentrations and TPMT activity which had previously been reported[9,10]. Red cell TPMT reflects the level of enzyme activity in other cells and tissues[11]. We report here an additional patient with pancytopenia related to abnormal azathioprine metabolism caused by TPMT deficiency.

## Case report

A 60-year-old woman presented with a 2-month history of extensive oral ulceration, and a 2 week history of a generalized mildly itchy skin rash. The oral ulceration was so severe that it had limited her to a liquid diet for the 4 weeks before presentation. Examination revealed the cutaneous changes of acute lichen planus with a generalized papular eruption, particularly on the palms where lesions were vesicular, also involving the flexural aspects of the forearms, and the trunk. There was extensive involvement of the whole oral mucosa with widespread superficial and deep ulceration. The ulcers were most prominent on the buccal mucosa and palate, with less severe involvement of the tongue.

Her condition was considered sufficiently severe to warrant treatment with immunosuppressive therapy; azathioprine was commenced at a dose of 50 mg twice daily. A full blood count taken before starting azathioprine was normal (Figure 2).

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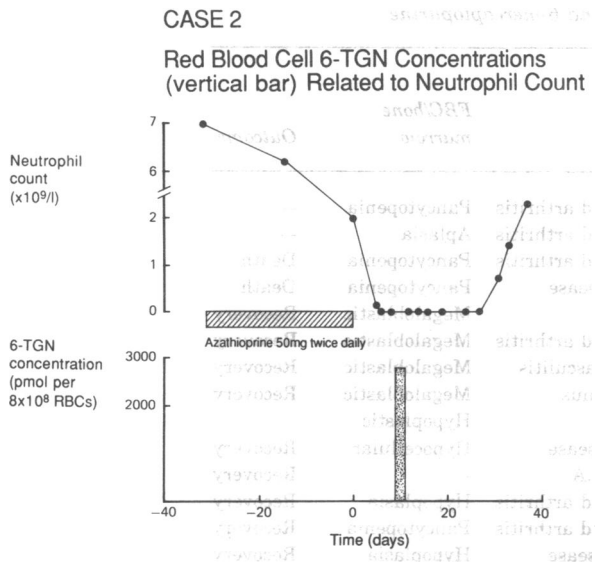


*Figure 2. Neutrophil count and 6-TGN level in relation to a 4 week course of azathioprine. This 6-TGN level is 10-fold higher than that measured in a control group taking long-term azathioprine with no history of myelosuppression[8]*

Two weeks later there had been a slight improvement in her condition. A repeat full blood count was normal and the dose of azathioprine was maintained at 50 mg twice daily. A full blood count taken 2 weeks later revealed a reduced white cell count of $2.6\times10^9$/l suggesting a degree of myelosuppression and azathioprine was therefore stopped. One week later the myelosuppression had worsened; the full blood count showed a haemoglobin of 10.5 g/dl, a white cell count of $0.8\times10^9$/l and platelet count of $94\times10^9$/l. Examination of the bone marrow aspirate revealed hypoplasia with megaloblastic changes. Granulopoiesis was reduced with giant metamyelocytes present; megakaryocytes were reduced. The changes were those of marrow hypoplasia presumed to be secondary to azathioprine treatment.

Red cell 6-TGN concentrations and TPMT activity were measured in a 5 ml blood sample in lithium heparin taken 13 days after azathioprine withdrawal but prior to any whole blood transfusions. Packed red blood cells were prepared and 6-TGN concentrations measured as previously described[12]. A Lysate for the assay of TPMT activity was prepared as described previously[13] and was stored at −80 °C. 6-TGN concentrations and TPMT activities were measured in laboratories in England and the United States of America respectively. At the time the assays were performed, each group of investigators was 'blind' to the results obtained by the other.

6-TGNs measured in a blood sample obtained 13 days after azathioprine withdrawal was 2828 pmol/$8\times10^8$ RBCs (Figure 2). TPMT activities measured in RBC lysate were 0.2 U/ml packed RBCs. The 6-TGN concentrations measured were 10-fold higher than in control patients taking long-term azathioprine therapy with no history of myelosuppression[8]. Measurement of TPMT activities in a previous study[3] showed that 99.7% of the population have TPMT activities between 5.5 and 19.5 U/ml RBCs. The very low level of TPMT activity reported here corresponds to that measured in the 1 in 300 individuals who are homozygous for $TPMT^{L}$[3].

The pancytopenia persisted for 4 weeks and was accompanied by two episodes of septicaemia requiring intensive supportive treatment. This included blood and platelet transfusions and intravenous antibiotics. A repeat bone marrow aspiration was taken after 2 weeks and showed continued hypoplasia with mildly increased numbers of myeloid precursors suggesting early recovery. One week later the peripheral blood count reflected this recovery with a significant improvement in haemoglobin, white cell and platelet counts. These changes were accompanied by an improvement in the clinical condition of the patient who was subsequently discharged home 4 weeks after admission to hospital. The lichen planus had improved progressively over this period and only minor ulceration persisted on the buccal mucosa at the time of her discharge.

Both oral and cutaneous lichen planus cleared completely within 8 weeks of commencing the 4-week course of azathioprine. Erosive oral lichen planus slowly recurred but never subsequently deteriorated to pre-treatment severity. Treatment with cyclosporin A was then commenced, initially as a mouth-wash but without therapeutic response, and then systemically at a dose of 4 mg/kg/day and resulted in a progressive symptomatic and clinical improvement. Following a 6 month course of systemic cyclosporin the oral ulceration had improved significantly but had not resolved completely.

## Haematological side effects reported for azathioprine

Reports of haematological toxicity with azathioprine are varied and include macrocytosis[14], anaemia[15], thrombocytopenia[16], leucopenia (Table 1) and pancytopenia (Table 2). Leucopenia is the commonest adverse haematological effect associated with the administration of azathioprine with an incidence in published large series (Table 1) which varies from 5%[23] to 25%[18,19] with a number of reports between these extremes. This could suggest intermediate TPMT activity with a $TPMT^{L}/TPMT^{H}$ genotype for these patients. Reports of severe marrow suppression and pancytopenia associated with azathioprine are few and often poorly documented. We are aware of

*Table 1. Azathioprine and 6-mercaptopurine treatment: incidence of leucopenia and pancytopenia/severe myelosuppression*

| *Study* | *No of patients* | *Disease* | *Percentage of patients with leucopenia* | *Cases of pancytopenia or severe myelosuppression* |
|---|---|---|---|---|
| Kolle 1969[17] | 30 | Juvenile rheumatoid, Stills | 17% | 3 |
| Lorenzen 1969[18] | 40 | Various | 25% | 4 |
| Ginzler 1975[19] | 73 | SLE | 8% | 6 |
| Singleton 1979[20] | 59 | Crohn's disease | 15% | 0 |
| Pollak 1980[21] | 160 | Renal transplant | 25% | 0 |
| Present 1980[22] | 68 | Crohn's disease | 10% | 2 |
| Mertens 1981[23] | 300 | Various | 5% | 4 |
| Hass 1982[24] | 56 | Multiple sclerosis/ Myasthenia gravis | — | 0 |
| Hall 1985[25] | 34 | Renal transplant | 9% | 3 |
| Kissel 1986[26] | 64 | Various, neurological | 22% | 1 |
| Kvein 1986[27] | 32 | Juvenile rheumatoid | | 2 |
| Hohlfeld 1988[28] | 105 | Myasthenia gravis | 16% | 3 |

*Table 2. Reported cases of severe myelosuppression associated with azathioprine and 6-mercaptopurine*

| Study | Duration of of prior treatment | Time to recovery | Drug | Daily dose | Sex | Age | Condition | FBC/bone marrow | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| Denman *et al.* 1970[29] | 27 weeks | — | Aza | — | M | 39 | Rheumatoid arthritis | Pancytopenia | — |
| | 11 weeks | — | Aza | — | F | 48 | Rheumatoid arthritis | Aplasia | — |
| | 12 weeks | — | Aza | — | F | 66 | Rheumatoid arthritis | Pancytopenia | Death |
| O'Donaghue 1978[30] | 11 years | 4 weeks | Aza | 2 mg/kg | M | 28 | Crohn's disease | Pancytopenia | Death |
| Taguchi *et al.* 1980[31] | 10 days | — | Aza | 100 mg | F | 26 | ITP | Megaloblastic | Recovery |
| Bacon *et al.* 1981[32] | 10 months | 1 week | Aza | 75 mg | F | 26 | Rheumatoid arthritis | Megaloblastic | Recovery |
| Lennard *et al.* 1984[33] | 2 months | 13 days | Aza | 1.7 mg/kg | M | 57 | Livedoid vasculitis | Megaloblastic | Recovery |
| Maddocks *et al.* 1986[7] | 4 weeks | 4 weeks | Aza | 1.7 mg/kg | F | 55 | Lichen planus | Megaloblastic Hypoplastic | Recovery |
| Goadsby *et al.* 1986[33] | 2 weeks | 8 days | 6MP | 1.5 mg/kg | F | 20 | Crohn's disease | Hypocellular | Recovery |
| Kvein *et al.* 1986[27] | 5 weeks | — | Aza | 2.5 mg/kg | M | — | Juvenile R.A. | — | Recovery |
| Verhelst *et al.* 1987[34] | 6 weeks | 8 weeks | Aza | 1.5 mg/kg | F | 61 | Rheumatoid arthritis | Hypoplasia | Recovery |
| Jeurissen *et al.* 1988[16] | 3 weeks | 3 weeks | Aza | 100 mg | F | 36 | Rheumatoid arthritis | Pancytopenia | Recovery |
| Lennard *et al.* 1989[8] | 26 days | 3 weeks | Aza | 2.6 mg/kg | M | 62 | Crohn's disease | Hypoplasia | Recovery |
| | 10 weeks | 4 weeks | Aza | 1 mg/kg | F | 63 | Rheumatoid arthritis | Pancytopenia | Recovery |
| | 21 days | 3 weeks | Aza | 2.3 mg/kg | M | 38 | Multiple sclerosis | Hypocellular | Recovery |
| Nossent *et al.* 1990[35] | 2 months | 12 days | Aza | 2.5 mg/kg | F | 40 | SLE | Pancytopenia | Recovery |
| Present report | 4 weeks | 4 weeks | Aza | 1.4 mg/kg | F | 61 | Lichen planus | Megaoloblastic Pancytopenia | Recovery |

17 detailed reports of marrow aplasia or pancytopenia associated with azathioprine or 6-mercaptopurine including the present report (Table 2). One report included assessment of xanthine oxidase activity which showed no impairment of this enzyme activity[33]. Cases in which allopurinol was taken concurrently with azathioprine have been excluded, as they almost certainly represent overdosage of azathioprine in the presence of impaired xanthine oxidase activity[16,32]. In 12 of these 17 cases, onset of myelosuppression occurred shortly after azathioprine (or 6MP) was started, with a range of 8 days to 10 weeks (mean 34 days). In five of these 12 cases RBC 6-TGNs were measured and were greatly elevated; the highest levels seen exceeded the upper limit of the normal range for patients on maintenance azathioprine by a factor of 14. An additional report[15] detailed early onset severe megaloblastic anaemia without thrombocytopenia, in which 6-TGNs were also very high nearly four weeks after withdrawal of azathioprine. Thiopurine methyl transferase activity was measured in 6 of these 12 cases and found to be absent or very low in all cases. It is possible that the other 6 cases of azathioprine-related early myelosuppression also have absent or very low TPMT activity, and a $TPMT^{L}/TPMT^{L}$ genotype. The five cases of azathioprine-related late myelosuppression may represent heterozygotes with intermediate TPMT activity, and $TPMT^{L}/TPMT^{H}$ genotype.

There is wide variation in the incidence of pancytopenia or serious myelosuppression in published large series of patients treated with azathioprine, with some authors reporting no such complications[20,21,24] whilst others have reported a surprisingly high incidence[17,18]. The largest of these series[23] included 300 patients with myasthenia gravis in whom severe leucopenia occurred in 16, pancytopenia in two and a severe megalocytic alteration in blood count in two. The 28 reports of severe myelosuppression or pancytopenia in these 12 series only included brief clinical details, but outcome was usually stated. There were four deaths from pancytopenia, all in the series reported by Lorenzen *et al.*[18]. Dosages of azathioprine used in these series did not exceed the recommended range and cases of myelosuppression appear to represent sensitivity to azathioprine rather than overdosage.

## Recording of adverse reactions to azathioprine

The Wellcome database of adverse events reported in association with Imuran® (Wellcome brand azathioprine) shows a total of 90 reports of bone marrow suppression world-wide (Personal communication, March 1991; Wellcome Group Clinical Safety Surveillance Service). Of these, 35 cases were stated to have been receiving treatment for less than three months at the time the adverse event occurred and one reporter speculated that the exceptional sensitivity to azathioprine may have represented TPMT deficiency. Sixteen of the 90 patients died, eight of them after less than 3 months' treatment, but in seven of the 16 fatal cases the available information suggested that death was not a consequence of marrow suppression per se.

The Committee on Safety of Medicines have received 25 reports of severe bone marrow supression related to azathioprine since July 1963 (Committee on Safety of Medicine - personal communication, April 1991). These comprise nine reports of pancytopenia, five of marrow depression, one of marrow aplasia and 10 of aplastic anaemia. In 10 of these 25 reports the outcome was death. In addition there were 19 reports of leucocyte suppression with 11 reports of agranulocytosis, five of granulocytopenia and three of leucopenia. In eight of these 19 reports the outcome was death. In the context of the widespread use of azathioprine for over 20 years these reports suggest either a very low incidence or under-reporting of severe haematological side effects.

## 'Idiosyncratic' reactions to azathioprine

Weinshilboum and Sladek's detailed paper on mercaptopurine pharmacogenetics[3] published in 1980 proposed that inherited variation in TPMT activity may represent one factor in individual variations in sensitivity to thiopurine drugs. In the

absence of alternative explanations to account for 'idiosyncratic' reactions to conventional doses of azathioprine and 6-MP it is surprising how frequently authors have overlooked this important paper. Whisnant *et al.*[36] published a retrospective review of the literature to determine the frequency of haematological side effects for rheumatoid arthritis patients treated with azathioprine. No comment was made as to the possible mechanism of severe azathioprine toxicity reported in this study. Lawson *et al.*[37] reviewed the adverse effects of azathioprine in 1984 and stated that the onset of bone marrow suppression in azathioprine recipients was 'predictable and dose-related'. Despite the availability at that time of a number of publications detailing idiosyncratic reactions to azathioprine the authors only referred to a series by Haas and Patzold[24] in which there were no major haematological side effects. Decker *et al.*[38] offered no comments on the mechanism of bone marrow suppression in their case report of an idiosyncratic reaction to azathioprine. In two recent case reports with literature reviews 20 cases of pancytopenia related to treatment with azathioprine were compiled[16,35]. No comment was made in either report as to the possible mechanism of bone marrow toxicity in these cases other than acknowledging the contribution of concurrent treatment with allopurinol in some of the cases.

### Azathioprine dosage

The recommended dose for azathioprine is 2-2.5 mg/kg daily. It is our impression that lower dosages are commonly prescribed and only increased in the absence of a therapeutic response. The two cases of azathioprine induced myelosuppression reported from our department both had very low/undetectable TPMT activity ($TPMT^L/TPMT^L$ genotype) and illustrate how early and severe haematological side effects may occur despite usage of azathioprine below the recommended dosage. The high frequency of leucopenia occurring in azathioprine treatment series (Table 1) is likely to be contributed to by patients with intermediate TPMT activity ($TPMT^L/TPMT^H$ genotype). Conversely, patients with high TPMT activity ($TPMT^H/TPMT^H$ genotype) metabolize azathioprine more rapidly to inactive metabolites and some of these may be underdosed at 2-2.5 mg/kg daily as has been reported with 6-MP in the treatment of lymphoblastic leukaemia[10]. Thus on the basis of TPMT pharmacogenetics[3] present dosage recommendations for azathioprine may be dangerous for one in 300 patients, moderately toxic to 11% and therapeutically ineffective in those patients with TPMT activities at the upper end of the $TPMT^H/TPMT^H$ range.

### Monitoring for azathioprine toxicity

The two cases now reported from our department show that blood counts are a poor method of detecting early azathioprine toxicity; changes in blood counts are delayed for a number of days after bone marrow suppression has occurred whilst the haematological reserve is exhausted. This azathioprine toxicity could have been predicted with a knowledge of the patients' inherited level of TPMT activity. Late onset myelosuppression has a more gradual onset and can therefore safely be detected by changes in blood counts[39]. The frequent monitoring of toxic effects throughout therapy, ie full blood counts, are recommended, even for those patients on constant dose, long-term treatment[40].

### Conclusion

There is now substantial evidence to suggest that individual variations in activity of TPMT may partly explain the variable response to azathioprine both for side-effects and therapeutic efficacy. This enzyme deficiency occurs at a frequency of one in 300 and can be detected before treatment with azathioprine is started[3]. A review of major series on the use of azathioprine has revealed serious haematological side effects occurring more frequently than one in 300, but such reports to The Committee on Safety of Medicines and the manufacturers of azathioprine, Wellcome, are very infrequent in relation to the widespread use of this drug. There are a number of factors which may explain this apparent discrepancy, but we consider the most important is likely to be under-reporting by physicians. The 11% of the population of the heterozygous ($TPMT^L/TPMT^H$) genotype will be more sensitive to azathioprine induced myelosuppression during a course of treatment than the 89% of subjects with high enzyme activity. Accumulating evidence now suggests that many cases of azathioprine toxicity are related to the inherited activity of TPMT. We have added a further case of early and severe myelosuppression associated with azathioprine and demonstrated again the inverse relationship between levels of the active metabolites 6-TGNs and activity of the catabolic enzyme TPMT. The indications for treatment with azathioprine should now be reviewed, safety monitoring reassessed and the question of pre-treatment screening of TPMT activity considered.

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